PHARMACOLOGICAL PROPERTIES OF HALO-DERIVATIVES OF BENZOBAMYL

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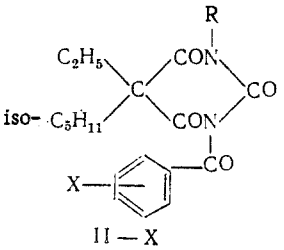
Benzobamyl (5-ethyl-5-isopentyl-3-benzoylbarbituric acid) (I) is an anticonvulsant drug of low toxicity, effective in the treatment of diencephalic and symptomatic epilepsy with generalized convulsions [3, 7]. It was subsequently found that (I) is an antihypoxic agent, a mild inducer of multipurpose liver oxygenases, and has a liver protectant effect in intoxication with CCl_4 [6]. Bearing in mind that the introduction of halogen into the benzoyl radical in benzonal and into benzyhydrylureas similar in structure to (I) results in an increase in their pharmacological activity [2], we have examined the anticonvulsant, antihypoxic, and hepatoprotectant properties of derivatives of (I) containing halogens in the benzoyl radical (II-X) in order to assess the effects of structural features of barbiturates on their activity.



R = H (II—X), X = o-F (II), m-F (III), p-F (IV), o-Cl V), m-Cl (VI), p-Cl (VII), o-I (VIII), m-I (IX), p-I (X)

EXPERIMENTAL (CHEMISTRY)

In a three-necked flask were placed 0.2 mole of 5-ethyl-5-isopentylbarbituric acid, 0.2 mole of the appropriate halobenzoyl chloride, and 0.2 mole of pyridine. The reaction was carried out with constant stirring at 100-125°C for 4.5-6 h. The course of the reaction was followed chromatographically every hour (benzene-methanol, 9:1).

When the reaction was complete, the mixture was cooled to 70°C, 50-60 ml of solvent added, stirred thoroughly, transferred to a beaker, and allowed to crystallize. The solid which separated was filtered off and washed with dilute (1:3) hydrochloric acid, followed by water until neutral. The solid was pressed thoroughly on the filter, and dried. The resulting halo-5-ethyl-5-isopentylbarbituric acids (II-X) were purified by recrystallization from toluene,propan-2-ol, or ethanol, and were obtained as colorless, crystalline solids. They were identified by their elemental analyses and IR spectra (Table 1).

EXPERIMENTAL (BIOLOGY)

Tests were carried out on 300 white mice of both sexes, weighing 22-25 g, and 100 male rats weighing 200-220 g. The compound (I) and its halo-derivatives were administered intraperitoneally to mice as suspensions in 1% starch mucilage, either once 2 h before application of the convulsant agent or hypoxic agent, or for six days simultaneously with 10 ml/kg of a 10% solution of CCl_4 in olive oil. The rats received the derivatives of (I) over four days together with 2.5 mg/kg of a 50% solution of CCl_4 in oil. Anticonvulsant activity was assessed in mice with convulsions induced by maximum electrical shock [11] or corazole [9]. In the first case, the halo-derivative of (I) was administered in doses of 100-900 mg/kg in order to calculate the ED_{50} values, and the survival and number of animals in which convulsions did not develop were assessed. In the corazole test, the drugs were administered in a dose of

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TABLE 1. Physicochemical Properties of Barbiturates (II-IX)

Compound	Reaction temperature, °C	Reaction time, h	Solvent for isolation and purification	mp, °C	Calculated, N, %	Found, N, %
II	110	5,5	Toluene	155—156	8,05	8,43
III	110	5,0	Propan-2-ol	132—133	8,05	7,72
IV	110	5,0	» »	115—116	8,05	8,42
V	125	6,0	Benzene, ethanol	110—111	7,67	8,04
VI	125	6,0	The same	125—126	7,67	7,80
VII	125	6,0	» »	140—142	7,67	7,83
VIII	100	5,0	Ethanol	140—141	6,14	6,62
IX	100	4,5	» »	159—160	6,14	5,78
X	100	5,0	» »	222—223	4,08	4,55

TABLE 2. Anticonvulsant and Hepatoprotectant Activity of Halo-Derivatives of Benzobamyl (M ± m, mean of 6-12 measurements)

Compound	ED_{50} in maximum electric shock, mg/kg	Corazole convulsion threshold		Duration of hexobarbitone sleep, min	Extent of fatty degeneration of liver, points
		mg/kg	anticorazole index		
I	78	667,3±45,5*	6,4	20,8±2,5*	2,8±0,2*
II	219	106,2±16,4	1,0	20,5±1,8*	2,7±0,3*
III	92	240,3±30,3*	2,6	21,3±4,3*	2,8±0,5*
IV	146	131,3±14,4	1,4	28,6±3,8*	3,5±0,4
V	652	133,0±4,4	1,3	23,1±2,9*	2,7±0,3*
VI	840	130,8±6,6	1,3	27,6±4,2*	2,7±0,3*
VII	870	109,4±4,3	1,1	33,7±4,2	3,6±0,4
VIII	102	202,9±22,6*	2,2	22,7±5,1*	3,3±0,2*
IX	186	127,7±16,0	1,4	37,9±8,1	4,2±0,5
X	132	106,9±12,9	1,2	38,3±4,3	3,9±0,3

*$P < 0.05$ compared to the control: the corazole convulsion threshold and CCl_4 hepatitis indices in untreated mice (duration of hexobarbitone sleep 40.5 ± 4.0 min, extent of fatty degeneration of the liver 4.5 ± 0.3 points).

100 mg/kg, and from the corazole convulsion threshold the anticorazole index was calculated as the ratio of the dose of the convulsant causing convulsions in mice protected by the derivatives of (I) and in those which did not receive the drug. Antihypoxic activity in the drugs in a dose of 200 mg/kg was assessed in model hemic (sodium nitrite, 250 mg/kg, subcutaneously), histotoxic (sodium nitroprusside, 25 mg/kg, subcutaneously), and hypoxic hypoxia with hypercapnia (250 ml pressure chamber) [4]. The hepatoprotectant activity of (I) derivatives in a dose of 100 mg/kg was calculated from their effects on the survival of the animals, retention of bromosulfalein, the duration of sleep induced by intraperitoneal injection of hexobarbitone (80 mg/kg), the weight coefficient, hepatic histoarchitectonics, necrosis, fatty dystrophy, the RNA, sulfhydryl group, and glycogen content of the hepatocytes [5], and the urokinase [1], alanine aminotransferase (ALT), aspartate aminotransferase (AST) [10], alkaline phosphatase [8] activity in the blood serum. The results were treated statistically using Student's parametric t criterion.

Results and Discussion. To judge from the ED_{50} values, the greatest anticonvulsant activity in the electrical shock test is shown by (I) and its derivatives containing fluorine in the meta-position (III) and iodine in the ortho-position (VIII) of the benzene ring. The para-fluoro (IV) and para-iodo-derivatives (X) are weakly active in preventing electrical convulsions. The active barbiturates in a dose of 100 mg/kg increased the survival of mice to 66.7-83.3%, and the number of animals in which fits did not develop to 33.3-50%. In the remaining compounds, the ED_{50} values were greater by a factor of 2.4-10.7 times than (I) in the same dose. Compounds (I), (III), and (VIII) had high anticorazole indices (Table 2).

In hemic and hypoxic hypoxia, the lifespan of the mice was significantly increased over the controls only by (I) (by a factor of 1.5, from 19.0 ± 0.8 to 29.0 ± 2.9 min and from 33.0 ± 2.6 to 50.0 ± 6.0 min, respectively), in histotoxic hypoxia by (IV) (by a factor of 1.4, from 7.0 ± 0.7 to 10.0 ± 0.6 min) and the o-chloro derivative (V) (by a factor of 1.9, from 7.0 ± 0.7 to 13.0 ± 2.7 min).

TABLE 3. Effects of Benzobamyl and Its o-Fluoro-Derivative (II) on the Retention of Bromosulfalein and Serum Enzyme Activity in Rats with CCl_4 Hepatitis (M ± m, mean of 8-10 measurements)

Factor	Intact animals	CCl_4 hepatitis	Benzobamyl + CCl_4	II + CCl_4
Retention of bromosulfalein, %	2,2±0,4	11,1±1,0	4,6±0,7	4,8±0,5
Urokinase, μmole/liter·h	4,6±1,1	117,4±20,9	38,8±8,2	36,9±9,4
ALT, mmole/liter·h	0,72±0,071	6,2±0,1	1,9±0,4	2,0±0,3
AST, mmole/liter·h	1,8±0,2	5,6±0,2	3,7±0,4	4,1±0,5
Alkaline phosphatase, mmole/liter·h	8,3±0,4	21,0±1,3	15,5±1,6	17,0±0,9

Note. Statistically significant changes ($P < 0.05$) are shown: for CCl_4 in comparison with intact animals, and for benzobamyl and (II) in comparison with CCl_4.

In the tests on mice, CCl_4 reduced the survival to day 7 to 70%, lengthened hexobarbital sleep by a factor of 1.8 (to 40.5 ± 4.0 min from the normal value of 22.1 ± 4.5 min), increased the liver weight coefficient by a factor of 1.4, and caused parenchymal steatosis extending along the lobule (from semiquantitative measurements). Administered together with the CCl_4, (I) protected all the experimental animals from a lethal outcome, reduced to normal the duration of hexobarbitone sleep, and reduced fatty dystrophy of the hepatocytes without changes in the weight coefficient of the liver. In its hepatoprotectant effects, the ortho-fluoro derivative (II) was not inferior to (I) following administration of CCl_4. Compounds (III) and (IV) increased survival to 75 and 84%, respectively, accelerated microsomal oxidation of hexobarbitone [(III) to a lesser extent than (I), (II), and (IV)]; (III) protected the liver from accumulation of neutral fat, while (IV) was inactive in this respect. In the group of compounds containing chlorine or iodine, (V), (VIII), and the meta-chloro derivative (VI) protected the liver from the toxic effects of CCl_4. The remaining derivatives were therapeutically ineffective (Table 2).

CCl_4 hepatitis in rats follows an especially severe course. In the intoxicated animals, biotransformation of hexobarbitone is considerably reduced, retention of bromosulfophthalein is increased by a factor of 5, discomplexation of the hepatic gullies occurs together with lymphoid-histiocytic infiltration and steatosis of the parenchyma, and the cytoplasmic RNA, sulfhydryl group and glycogen content of the hepatocytes is reduced. The number of necrotized hepatocytes reaches 5.9% (normal value 0.9%). The toxicant-induced cytolysis results in an increase in the permeability of the membranes and passage of hepatic enzymes. For example, urokinase activity increases 25 times, ALT 8.6 times, AST 2.8 times, and alkaline phosphatase 2.5 times.

The most active compounds, (I) and (II), counteract the extension of hexobarbitone sleep, retardation of the secretion of BSF, infringement of the architectonics of the liver, and normalize the parenchymal histochemical factors, including the neutral fat content. On therapy with (I), the necrotized hepatocytes comprise 2.5%, and with (II), 2.7%. In the blood serum, the urokinase activity is only eight times, and the ALT, AST, and alkaline phosphatase activity 2-2.8 times greater than the figures for intact rats (Table 3).

Hence, the most active compounds in preventing mice from convulsions when subjected to an electric current (a model of generalized epileptic convulsions) are (I), (III), and (VIII). These same compounds show high antagonism to corazole, modeling absentia epileptica. In the remaining barbiturates, the considerable differences in the ED_{50} values in maximum electric shock were not accompanied by changes in the anticorazole index, which remained approximately constant, at a low level. Antihypoxic activity in histotoxic hypoxia induced by sodium nitroprusside, which is converted into cyanide, was shown by (I), (IV), and (V) only, and in addition (I) increased the survival of mice in hemic hypoxia under conditions of rapid methemoglobin formation.

Therapeutic activity in CCl_4 hepatitis was highest in the ortho-derivatives (II), (V), and (VIII), the meta- and para-isomers being less active (ortho > meta > para). The hepatoprotectant activity of the chloro- and iodo-compounds was lower than that of the fluoro-compounds, especially in the hexobarbitone sleep test. The type of halogen has a considerable effect on hepatoprotectant activity in the meta- and para-isomers, but a lesser effect in the o-derivatives. This suggests that steric factors are not responsible for the variations in the hepatoprotectant activity of the barbiturates (II-X), depending on the halogen present.

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HYDRAZIDES AND SULFONYLHYDRAZIDES OF THE QUINOLINE SERIES AS INHIBITORS OF SERUM AMINOOXIDASE

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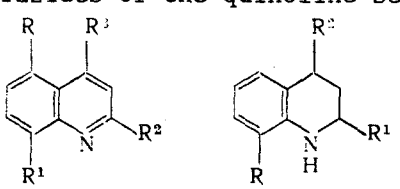
Human serum aminooxidase (AO) is an important enzyme [1], interference with the activity of which is seen in a variety of pathological states, such as thermal burns [12], chronic cirrhosis and fibrosis of the liver [10, 11], and some gynecological conditions [9].

An important task of theoretical and practical medicine is to find compounds having activity on this enzyme.

The AO inhibitors cuprizone, sodium azide, and isoniazid, which retard the AO-catalyzed deamination of benzylamine, block AO activity only at relatively high concentrations (on average $I_{50} = 10^{-3}$ M) [1, 8].

We here report the synthesis and antiaminooxidase activity of some novel AO inhibitors, namely hydrazides and sulfonylhydrazides of the quinoline series.



I, II, VI — X III — V

R=SO_2NHNH_2 (I, II), H (IV — VIII, X), $CONHNH_2$ (III, IX); R^1=OMe (I), Me (II, IX), H (III, IV, VI, VII), $CONHNH_2$ (V, VIII), SO_2NHNH_2 (X); R^2=H (I — III, V, VII — X), $CONHNH_2$ (IV, VI); R^3=H (I, II, VI, VIII — X), $CONHNH_2$ (VII).

8-Methoxy- and 8-methyl-5-quinolinesulfonhydrazides (I, II) were obtained by treating 8-methoxy- and 8-methyl-5-quinolinesulfonyl chloride [4, 5] with hydrazine hydrate in chloroform.

Reduction of 8-, 4-, and 2-quinolinecarboxylic acids with nickel-aluminum alloy (Raney alloy) in 10% NaOH gave 1,2,3,4-tetrahydroquinoline-8-, -4-, and -2-carboxylic acids, which were converted into their ethyl esters [12] by boiling with ethanol in the presence of sulfuric acid and hydrogen peroxide, as described in [16]. The ethyl esters, on boiling with hydrazine hydrate in absolute ethanol, were converted into 1,2,3,4-tetrahydroquinoline-8-, -4-, and -2-carbohydrazides (III-V). The properties and yields of the compounds obtained are given in Table 1. 2-, 4-, 8-, and 8-methyl-5-quinolinecarbohydrazides (VI-IX) [2, 7, 15,

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